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DEVELOPMENT OF A PHYSIOLOGICAL COLLAGEN ARCHITECTURE THROUGH ANISOTROPIC NANOFIBROUS POLYCAPROLACTONE-COLLAGEN SCAFFOLD FOR CARTILAGE TISSUE ENGINEERING

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SUMMARY: *In this study, we propose an innovative 3D electrospun scaffold with distinct biomimetic zones capable of both simulating the collagen fibrous architecture of the native cartilage and presenting mechanical features that show potential to be compatible with both static and dynamic cell culture protocols.*

1 INTRUDUCTION

The main goal of cartilage tissue engineering (TE) is the *in vitro* recreation of the depth dependent nanostructural organization of the fibrous collagen network that comprises the cartilage natural extracellular matrix. In fact, native cartilage is anatomically and functionally divided into three nanofibrous zones with distinct mechanical properties due to fiber size and orientation, progressing from perpendicular to the subchondral bone surface in the deepest zone, to random in the middle zone and to parallel in the superficial region.

Though the promising results of both fibrous and porous scaffolds used to overcome this challenge during the past few years [1], none of the followed methodologies is currently able to guarantee an optimal balance between biological features, mechanical properties and suitable topographic cues. Additionally, recent studies have indicated the necessity to include mechanical stimulation via a bioreactor in the scaffolding strategy in order to enhance the modulation of the chondrocyte behavior *in vitro* [2].

Taking all this into account, we have developed a newly 3D scaffold capable of not only simulating each cartilaginous zone with great morphological accuracy, but also providing a biocompatible porous network suitable for both static and dynamic cell culture protocols.

2 METHODOLOGY

To recreate each cartilaginous zone, different electrospinning set ups, including a rotating mandrel and an ethanol-water bath as collectors, were used to fabricate Polycaprolactone (PCL) nanofibres with controlled size and alignment. In a second stage, the fibrous biomimetic zones were assembled within a collagen hydrogel that after a lyophilisation process was able to physically support the fibres inside a heterogeneous porous network.

The individual parts of the 3D PCL-collagen scaffold were characterized via SEM analysis and their mechanical properties were evaluated via static and dynamic (via a bioreactor) compressive and tensile tests.

3 RESULTS AND DISCUSSION

As it is illustrated in Fig. 1, the design and fabrication methods adopted were able to successfully incorporate three biomimetic layers of PCL nanofibres within a collagen porous network.

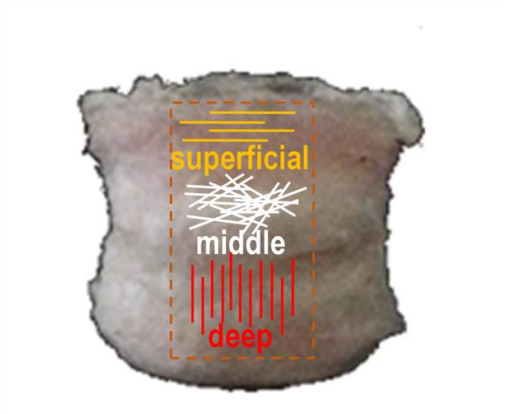

Fig. 1. 3D PCL-collagen scaffold: scheme of the PCL biomimetic zones assembled inside the collagen porous network

SEM analysis (Figs. 1, 2 and 3) has confirmed that each PCL fibrous layer shows analogous architecture and topography relatively to its native counterpart due to the precise modulation of the fibre orientation via electrospinning. Relatively to the mechanical properties and size of the electrospun nanofibres (Tab. 1), the three zones presented Young moduli and diameters suitable for protocols involving cartilage TE strategies [3]. These preliminary results suggest that the presented scaffold can offer an enhanced cellular microenvironment capable of promoting cartilage regeneration.

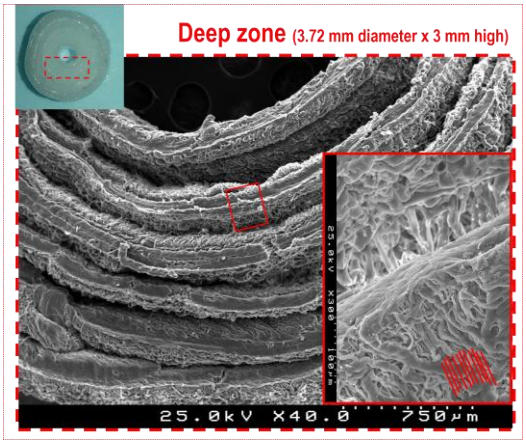

Fig. 2. Deep zone: SEM analysis.

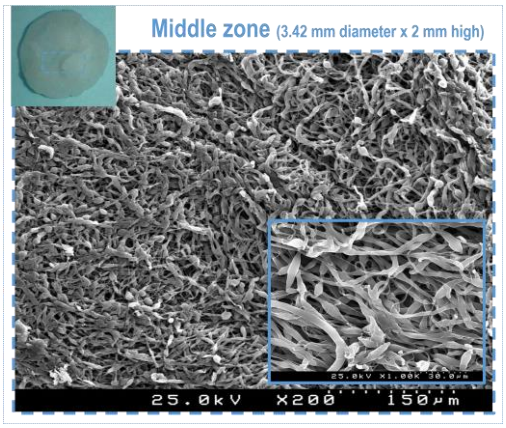

Fig. 3. Middle zone: SEM analysis.

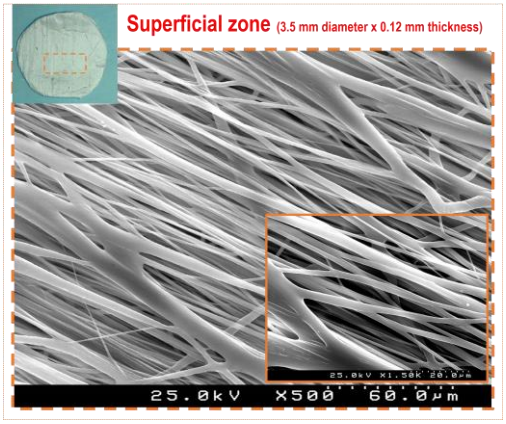

Fig. 4. Superficial zone: SEM analysis.

Tab. 1 Properties of the electrospun fibres

Zone	Diameter (µm)	Young M. (kPa)
Deep	2.8 ± 1.8	192.4 ± 8.2
Middle	4.8 ± 2.6	22.6 ± 6.7
Superficial	2.8 ± 1.8	1100 ± 100

4 REFERENCES

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